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Genetic studies on the *Wx* gene of sorghum *(Sorghum bicolor* (E.) Moench.]

I. Examination of the protein product of the waxy locus

Jaw-Shu Hsieh

Department of Agronomy, National Taiwan University
Taipei, Taiwan, Republic of China

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Abstract. The waxy locus on the sixth linkage group in sorghum *[Sorghum bicolor* (L.) Moench.] controls the production of amylose in the endosperm. Six sorghum lines were used in the present study to examine the variation in quantitative level of the ***Wx*** gene product by SDS-PAGE. A 60kD protein was found among nonwaxy sorghum lines as well as near-isogenic lines with *W x* gene. It seems to suggest that the ***Wx*** gene codes for *W x* protein and, as a consequence, amylose content in endosperm starch, and the waxy phenotype is characterized by loss of this protein. The amount of ***Wx*** protein increased with increasing *W x* gene dosage, though not linearly. The second dose seems to play more important role in increasing *Wx* protein than the third dose.

Key words: Dosage effect; SDS polyacrylamide electrophoresis; *Sorghum bicolor;* Waxy locus: *W x* protein

Introduction

The waxy endosperm was first discovered in maize (Zea mays) kernels and introduced into the West by a presbytarian missionary in Shanghai, China in 1908 (Collins, 1909). Collins (1909) described the endosperm of this Chinese maize to be opaque, dull, and with cutting characteristic like a hard wax; hence, the term waxy was given. Genetic crosses between waxy and nonwaxy maize varieties resulted in F_1 with nonwaxy endosperm behaving as a simple Mendelian trait. The symbols *wx* (waxy) and *Wx* (nonwaxy) were adopted to designate the recessive and dominant alleles of the locus, respectively (Brink and MacGillivaray, 1924).

The *Wx* endosperm contains both amylose and amylopectin whereas *wx* endosperm contains primarily amylopectin (Wall and Blessin, 1969). On the enzymatic basis of the *wx* mutation, Nelson and Rines (1962) found that starch granules isolated from *wx* maize endosperm lacked the enzymatic activity which was required to synthesize amylose whereas this activity was present in *Wx* endosperm starch granules. This enzyme activity was also found in *Wx*, but not *wx*, pollen starch granules (Bryce and Nelson, 1978). In maize, it was found that *Wx* gene product (*W x* protein) was

starch-granule-bound nucleoside diphosphate (NDP) sugar-starch glucosyl transferase, since the transferase activity and the amount of a 60 kD protein increased with increasing dosage of the *Wx* gene (Tsai, *1974).* The waxy phenotype is characterized by a significant reduction of strach-granule-bound transferase activity as well as a loss of the *Wx* protein (Echt and Schwartz, *1981;* Nelson *et al., 1978;* Tsai, *1974).* This 60kD protein has also been detected in rice *(Oryza sativa)* grains (Sano, *1984).*

Waxy sorghum, another plant species with waxy endosperms, was first used in World War Ⅱ as a substitute for tapioca starch. The amylose/amylopectin content is considered to be one of the major determinants for brewing and cooking qualities for sorghum grains (Hsieh and Pi, *1979).* Normal or nonwaxy sorghum starch has approximately *75%* amylopectin and 25% amylose, while waxy sorghum starch contains nearly *100%* amylopectin (Sullins and Rooney, *1974).* The results of genetic studies demonstrated that the amylose content in sorghum endosperm was controlled by a simple Mendelian gene and the dosage of dominant allele (Hsieh and Pi, *1979).* In order to further examine the expression of *Wx* gene at protein level in sorghum endosperm, lines with waxy and nonwaxy endosperm and four pairs of near-isogenic lines were used to identify the gene product of *Wx* gene in sorghum.

Materials and Methods

Plant Materials

Six sorghum lines collected from various countries and preserved in our laboratory were used in the present investigation. In addition, four near-isogenic lines with different homozygous pairs of *Wx* and *wx* were also included. Near-isogenic lines were selected from a cross of nonwaxy variety (DDET 6662) and waxy variety (SA 6649). Different pericarp colors were selected from F_2 popluation. Only those plants with heterozygous waxy locus were selected. Heterozygous waxy locus

Table 1. *The endosperm types and origin of sorghum lines used for* ***Wx*** *gene studies*

Line code No.	Endosperm type	Origin
9 A	nonwaxy	a Shallu type from Australia
PI 257595	nonwaxy	a PI line from Taichung DAIS.
TAM 428 (SC 0110-9)	nonwaxy	a converted exotic line obtained from Kaoshiung DAIS.
Tx 403	nonwaxy	a combine kafir from Texas, USA.
SA 6649	waxy	a Feterita type from Kansas, USA.
129–3 A	waxy	a selected line from Kinsien No. 3 × IS-84, F_{15}
Ⅰ *Wx*(15405)	nonwaxy	
Ⅰ *wx*(15806)	waxy	selected from a cross of SA 6649 and DDET 6662, milky pericarp
Ⅱ *Wx*(1103)	nonwaxy	
Ⅱ *wx*(1901)	waxy	selected from a cross of SA 6649 and DDET 6662, milk pericarp with red sectors
Ⅲ *Wx*(12311)	nonwaxy	
Ⅲ *wx*(13901)	waxy	selected from a cross of SA 6649 and DDET 6662, brown pericarp
Ⅳ *Wx*(29013)	nonwaxy	
Ⅳ *wx*(18001)	waxy	selected from a cross of SA 6649 and DDET 6662, brown pericarp with red sectors

was identified by I_2-KI staining at the pollen stage. Single plants with heterozygous waxy locus have been propagated for 8 generations. The endosperm types and origin of sorghum lines used in the present investigation are shown in Table 1.

For the evaluation of dosage effect of *Wx* gene in gene product, reciprocal crosses were made within the same pair of near-isogenic lines to obtain lines with different dosage of *Wx* gene under the same genetic background. Table 2 shows grain characteristics and dosage of *Wx* gene in endosperms of reciprocal crosses within near-isogenic line I.

Table 2. *Grain characteristics and dosage of* ***Wx*** *gene in endosperms of crosses and reciprocal crosses within the near-isogenic line I*

Crosses	Genotype		Dosage of *Wx* gene in endosperm
	Embryo	Endosperm	
I Wx × *I Wx*	*WxWx*	*WxWxWx*	*3*
I Wx × *I wx*	*Wxwx*	*WxWxwx*	*2*
I wx × Ⅰ *Wx*	*Wxwx*	*Wxwxwx*	*1*
Ⅰ *wx* × Ⅰ *wx*	*wxwx*	*wxwxwx*	*0*

Isolation of Starch Granules

Starch granules were isolated from dried grains according to the method of Schwartz and Echt (1982). Briefly, 10 de-embryoed grains were homogenized in 1 ml of buffer A [0.055 M Tris-HCl (pH 6.8) containing 2.3% (w/v) sodium dodecyl sulfate (SDS) and 5% (v/v) β-mercaptoethanol]. The homogenate was filtered through four-layer cheesecloth and centrifuged at 7,000 xg for 1 min. The starch granule-containing pellet was washed with 1 ml of acetone for three times and was then lyophilized and stored in liquid nitrogen.

Protein Extraction

Protein was extracted from either individual grain or starch granules. Freeze-dried starch granules (5 mg) and individual grain were homogenized, respectively, with 50 μl of buffer **A** and heated in a boiling water bath for 5 min. After cooling in ice bath, the gelled solution was added with 100 μl of buffer A with gentle stirring. The slurry was centrifuged at 7,000 xg for 5 min. The resulting supernatants were immediately used for gel electrophoresis.

Gel Electrophoresis

The *Wx* protein in protein extract from individual grains or starch granules was identified by SDS-polyacrylamide electrophoresis (SDS-PAGE) (Laemmli, 1970). The gels used were 10% or 12.5% acrylamide and were stained with Coomassie Blue. Moleular weight was determined by co-migration of Sigma Molecular Weight Markers. Densitometric measurements of the stained gels were performed on a Soft Laser Scanning Densitometer (SLR-504-XL, Biomed Instruments, USA.).

Results

Figure 1 clearly shows that there are one major protein and four minor protein bands in high molecular weight region in protein extracts from single grain of four nonwaxy sorghum lines (9A, PI 257595, TAM 428, Tx 403, and Ⅱ *Wx*). The major protein band was estimated to have molecular weight of 60 kD. In contrast to nonwaxy lines, protein extract from single grains of two waxy lines (SA 6649 and 129-3A) contained no major protein band as visualized by SDS-PAGE (Fig. 1). Similar results were obtainned from proteins extracted from starch granules of four different pairs of near-isogenic lines (I wx, I *Wx*, Ⅱ *wx*, Ⅱ *Wx*, Ⅲ *wx*, Ⅲ *Wx*, *N wx*, and *NWx*; Fig. 2). A 60 kD protein was found in protein extract from starch granules of near-isogenic lines with *Wx*. However, no 60kD protein was present in

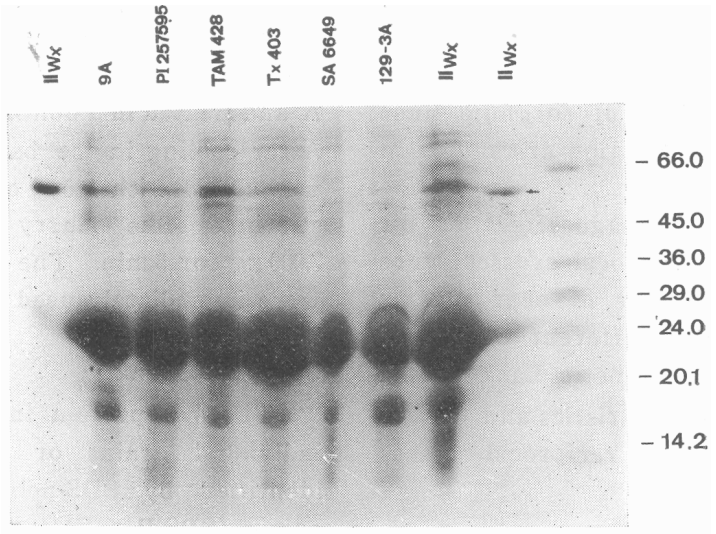

Fig. 1. Protein profiles from SDS polyacrylamide gel electrophoresis. From left to right, lanese 1 and 9 were proteins from starch granules. Lanes 2, 3, 4, 5, 6, 7 and 8 were proteins from individual grains. Arrow indicates the *Wx* protein.

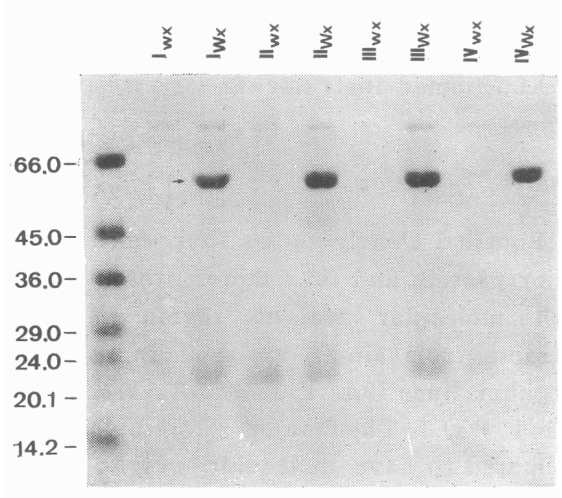

Fig. 2. SDS polyacrylamide gel electrophoretogram of proteins extracted from starch granules of four different pairs of near-isogenic lines. Arrow indicates the *Wx* protein.

protein extract from starch granules of near-isogenic lines with wx. The major protein band contained about 80% of total heat-extractable, starch-granule-bound proteins.

During fertilization in plant life cycle, one male gamete fertilizes the female gametic nucleus to form the diploid zygote, and the other male gamete combines with the diploid endosperm nucleus to form the triploid (diploid + haploid) tissue that will nourish the embryo. Thus, triploid endosperm tissue could be used to examine the effect of *Wx* gene dosage on

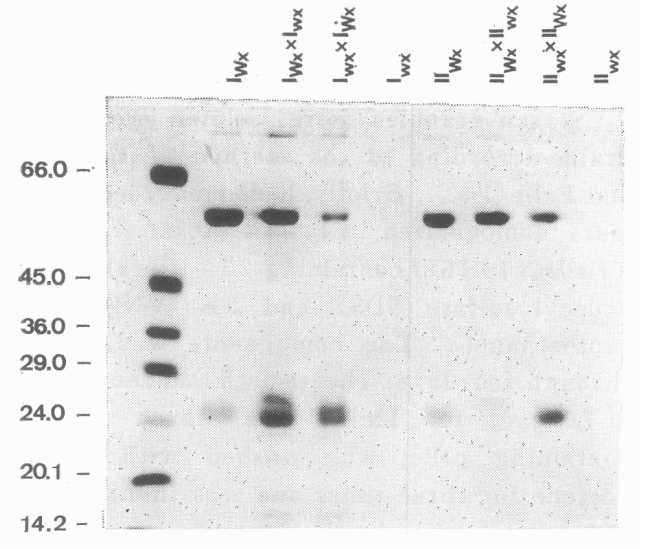

Fig. 3. Dosage effect of *Wx* gene on the *Wx* protein level. The number of *Wx* alleles are shown, in terms of cross combination, on the top of the gel.

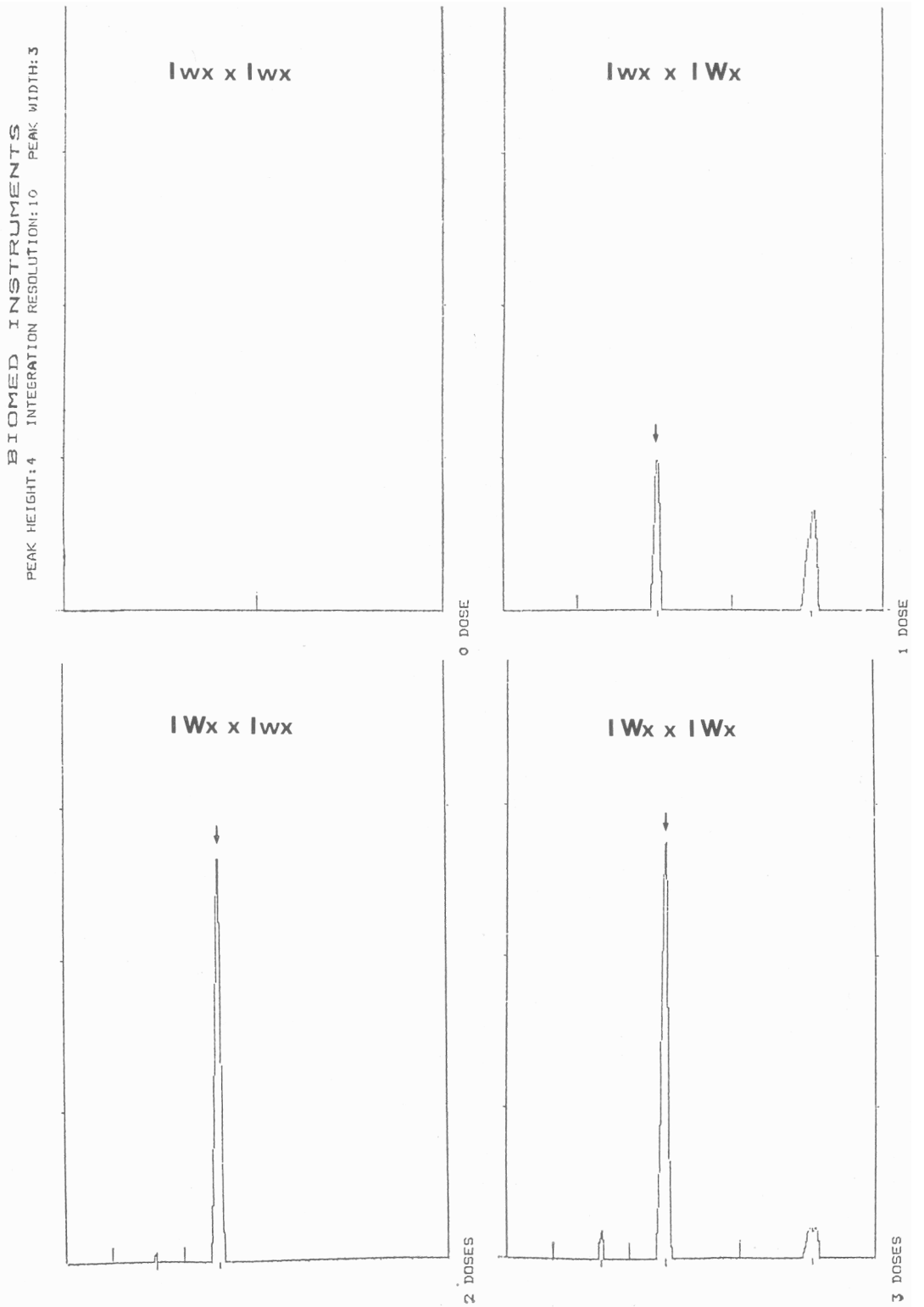

Fig. 4. Densitometric measurement of dosage effect of Wx gene on the *Wx* protein level. Arrows indicate the peak height of *Wx* protein.

the increment of the gene product by reciprocally crossing within the same pair of near-isogenic line. Two different pairs of near-isogenic lines, I *Wx*, I *wx*, and II *Wx*, II *wx*, were therefore chosen to make the dosage series and the hybrid grains of which were analysed for the level of *Wx* protein. The amount of *Wx* protein increased with increasing *Wx* gene dosage, though not linearly (Figs. 3 and 4). The amount of *Wx* protein, based on the measurement by densitometry as shown in Fig. 4, did not increase proportionally in accordance with the *Wx* gene dosage. It seems that the effect of the second *Wx* dose is more important than that of the third *Wx* dose in increasing the *Wx* protein level (Fig. 4). The other minor proteins of lower molecular weight did not vary with the *Wx* gene dose.

Discussion

The present study indicates that *Wx* gene of sorghum seems to encode a 60kD protein. This conclusion is based on the observations that (a) 60kD protein was only found in protein extract from nonwaxy grains, but not from waxy grains and (b) 60kD protein was present in starch granules of near-isogenic lines with *Wx* gene but absent in starch granules of near-isogenic lines with *wx* gene. It has been shown that *Wx* proteins of maize and rice had molecular weight of 60kD (Echt and Schwartz, 1981; Sano, 1984). Thus, 60kD protein found in the present investigation seems to be *Wx* protein in sorghum endosperm.

The waxy locus in maize and rice has been mapped on chromosome 9 and chromosome 6, respectively (Neuffer, 1968; Okuno, 1978). For sorghum, the waxy locus has been mapped on chromosome 6 (Karper *et al.*, 1934). The cross-over values between *ys2* (deep yellow lethal) and *Wx* and between *Wx* and bm (waxy bloom on leaf and sheath) were estimated to be 26 and 5, respectively (Karper *et al.*, 1934; Webster, 1965). Starch in homozygous *Wx* sorghum endosperm contained 20–35% amylose and 65–80% amylopectin, whereas the *wx* endosperm lacked amylose and contained primarily amylopectin (Hsieh and Pi, 1979). In our previous work, we showed that the amount of amylose in sorghum grains increased with increasing *Wx* gene dosage but not linearly (Hsieh and Pi, 1979). Furthermore, the second dose of *Wx* gene was found to have drastic effect in increasing the amylose content in sorghum grains. At present, we do not know the identity and function of 60kD protein. If 60 kD protein plays important role in regulating amylose synthesis as suggested in maize and rice, then no linear correlation between *Wx* gene dosage and the amount of 60kD protein is to be expected. Our present work indeed showed that the amount of 60 kD protein did not increase linearly with increasing of *Wx* gene dosage. In contrast to our results, the amount of *Wx* protein was reported to be highly correlated with *Wx* gene dosage in maize and rice (Echt and Schwartz, 1981; Sano, 1984; Tsai, 1974).

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蜀黍臘質基因的遺傳研究

一、臘質基因座的蛋白質產物之檢定

謝 兆 樞

國立臺灣大學農藝系

臘質基因座是位於蜀黍（俗稱高粱）第六對染色體上。它控制著種實胚乳中粉質澱粉 (amylose) 的合成。六種不同澱粉特性的品種（系）的胚乳，利用 SDS-PAGE 電泳分析結果，在種實粗抽出物中，非臘質品種（系）均較臘質者增加一個分子量爲 60 kD 的蛋白質。此蛋白質在純化的臘質基因近同源系胚乳澱粉顆粒的抽出物中，經電泳分析證實即爲非臘質基因 (*Wx*) 所調控合成，並與澱粉顆粒緊密結合，通常稱之爲 *Wx* 蛋白質。

利用臘質基因近同源之間的正反交配所得不同劑量的 *Wx* 基因的胚乳中，*Wx* 蛋白質的含量隨著 *Wx* 基因的劑量而增加；惟在增加 *Wx* 蛋白質含量的效應上，第二劑量的 *Wx* 基因較第三劑量來得顯著與重要。